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CLINICAL AND EXPERIMENTAL STUDY

OF THE

Leucocytosis of Diphtheria.

BY

JOHN LOVETT MORSE, M.D.

(*Reprinted from the Boston City Hospital Medical and Surgical Reports.*)

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By John Lovett Morse, M.D.

A great deal of work has been done during the last few years on the subject of leucocytosis, especially in acute diseases. Diphtheria, however, has been rather neglected. As it is an acute disease with marked constitutional symptoms due to the absorption of the toxic products of bacterial growth, a leucocytosis is to be expected. The observations hitherto reported have shown this to a marked degree.

Bouchut and Dubroisay[1] in 1877 made repeated examinations of the blood by Hayem's method, in 84 children sick with diphtheria, and obtained 4,305,000 as the mean of 93 counts of the red corpuscles; that is to say, a moderate diminution, which was the greater the severer the sickness and the higher the fever. The white cells were, in the average of 93 counts, increased to 26,824, making the ratio of white to red equal 1 to 160. Bouchut gives the following summary regarding the frequency of the occurrence of the various numbers. The white corpuscles amounted to:

0 to 5,000	in 1 case.
5,000 to 10,000	in 11 cases.
10,000 to 20,000	in 28 cases.
20,000 to 30,000	in 19 cases.
30,000 to 40,000	in 15 cases.
40,000 to 50,000	in 7 cases.
50,000 to 60,000	in 9 cases.
60,000 to 100,000	once in every 10 cases.

The very high grade of leucocytosis reported by these observers seems almost incredible, but is probably to be

* A paper to which was awarded the First Lyman Prize for the year 1894. From the athological laboratories of the Boston City Hospital and the Harvard Medical School.

explained by the fact that they used Hayem's apparatus, which does not compare in accuracy with the Thoma-Zeiss. It is also possible that owing to the lack of bacteriological examinations, there may have been some errors in diagnosis.

In a later communication Bouchut[2] reports the results of 177 observations in severe "septicemic diphtheria." He found an "acute leucemic" condition of the blood, which increased with the severity of the disease and diminished again with an improvement. He did not find this marked leucocytosis in the lighter cases, however. Hence he considers the examination of the blood as regards the number of the white corpuscles as of importance for diagnosis. No differential count of the white corpuscles was made in these cases, of course.

The only other examination of the blood in diphtheria which I have been able to find reported in an extended search through the literature of leucocytosis is one by Reinert.[3] He studied one case in a twenty-four-year-old girl, where a blood examination was made, on account of an ulcer of the stomach, before the attack of diphtheria. His results were as follows:

11, xii, 3 P.M. Red 4,772,000.
13, xii. Patient sickened with diphtheria with moderate fever.
16, xii, 3.30 P.M. Red, 5,050,000; white, 5,763; white to red as 1 to 870.
19, xii, 3.30 P.M. Second day after cessation of fever. Red, 4.398,000.
19, xii, 4.30 P.M. Red, 4,732,000; white, 7,700; white to red as 1 to 610.

That is, a slight increase of the colored elements was observed during the fever period, "which may be easily explained by the diminished ingestion of food and drink." There was little or no increase in the leucocytes.

As the results hitherto obtained were rather surprising and lacked confirmation, there seemed to be room for further investigation. Moreover, many points had not been studied, as the character of the leucocytosis, its influence on prognosis, and its relation to the membrane, glands, and complications. Through the kindness of Drs. Buckingham and Folsom I have been enabled to examine the blood of 30 cases in the diphtheria ward of the Boston City Hospital. Owing to the

lack of time, in but one case was more than a single examination made. The presence of the Klebs-Loffler bacillus had been previously demonstrated in all of them, and none of them were complicated by other diseases, acute or chronic. The effect of the symbiosis with other bacteria was not investigated.

The blood was in every case taken from the lobe of the ear and examined with a Thoma-Zeiss apparatus. It was diluted 1 to 200 with a three-per-cent. salt solution colored with methylene-blue, and the corpuscles in one cubic millimeter counted. Cover-slips were in all cases made at the same time, hardened by heat or benzine, and stained with Ehrlich's "triple-stain." A differential count of from 300 to 500 white corpuscles was then made, the classification recommended by Ehrlich being used. As far as possible, the blood was taken at the same time of day and between three and four hours after food. The condensed results of my work are appended in the accompanying table, which is arranged according to the day of the disease.

It is evident from the above table that diphtheria is accompanied by a marked hyperleucocytosis, larger even than that found in pneumonia. This has become well marked by the third day, and is probably present earlier. In a general way it increases as the disease progresses, is greatest at the height of the disease, diminishes during convalescence, and disappears with or soon after the membrane. It also in a general way corresponds with the amount of membrane, varying with it directly. There are notable exceptions to this rule, however, as in Case VII., where the throat was full of membrane and only 8,000 white corpuscles were found, and in Case V., where there were 42,000, but only a moderate amount of membrane. There seems to be no evident connection between the glandular enlargement and the increase in white corpuscles. It is noticeable, however, that the fatal "septic" cases with greatly enlarged glands all showed a very marked hyperleucocytosis. Some of the mild cases, nevertheless, with little or no enlargement of the glands, showed just as much. The condition of the lungs and kidneys apparently had no influence in determining the number of white cells. It thus becomes evident that the hyperleucocytosis is

not due to any symptom or combination of symptoms, but is the result of some general influence which is present in every case. This influence can be no other than that of the toxines absorbed. Whether the individual variation is due to the amount of absorption, to some difference in the virulence of the toxine absorbed, or to some difference in the resistant powers of the person attacked, must, in the present state of our knowledge, be left unanswered. In the great majority of cases the increase was in the polynuclear neutrophiles, that is, the typical leucocytosis. In several, however, the proportions closely approach the normal. In a few, chiefly convalescent cases, there seemed to be a lymphocytosis; this, too, in cases without much external glandular enlargement.

The number of the erythrocytes was, with one or two exceptions, always somewhat above normal. The only patient in which they were diminished was a woman, evidently subject to a chronic anemia. I am unable to give any explanation of this increase.

This study establishes the fact pretty clearly, I think, that the examination of the blood in diphtheria as to leucocytosis is of no value in prognosis. For, although fatal cases have a pretty marked one, yet it is almost always present, and often very pronounced, even in the mildest cases.

Having thus established the existence of a leucocytosis in diphtheria, I will now take up briefly the various views with regard to the origin of leucocytosis in general, and later detail the results of some experiments in animals which I have made in this connection. I will take up the theory which seems to me most probable last, that of Goldscheider, comparing his views with the others in turn and quoting largely from him.

Von Limbeck[4] considers that leucocytosis stands in the closest connection with the formation of exudate, and considers it as entirely secondary to the exudation. He found that when the toxic products of micro-organisms were introduced into animals, a reaction followed which corresponded to the size of the dose; that neither blood nor other tissues reacted to small amounts; and that after larger, a local reaction and proportionate hyperleucocytosis occurred. A much more significant reaction of the tissues and a greater

hyperleucocytosis followed when living micrococci were introduced. He concludes that the hyperleucocytosis arises from the development or presence of bacterial products in the organism, which have a further action on the white cells and bring them into the circulation. He does not explain what this action is, and expresses no opinion as to whether those cells are new-formed or merely driven out of the blood-forming organs. He nevertheless calls attention to chemotaxic action as probably playing a rôle in the process.

His theory must be considered as of little value, however, as he did not recognize the hypoleucocytosis which has been found by other observers. His failure to find this was undoubtedly because he made no examination under forty-eight hours. Moreover, other investigators, notably Goldscheider and Jacob, have produced a marked hyperleucocytosis by the subcutaneous injection of organic extracts without producing any swelling or exudate. These experiments evidently destroy the foundations of von Limbeck's theory.

Römer,[6] as the result of his own and Buchner's[7] work, concludes that the destructive products of dead bacteria or other cell-life, when introduced into the circulation, exert a direct formative action on the white cells, causing a new formation of leucocytes, exclusively in the venous blood. This increase arises through amitosis. He also claims that the cells found increased may in part be already present in the blood-making organs and be brought into the circulation by chemotaxic action. Rieder[8] and Löwit completely contradict his experiments and arguments, while Goldscheider and Jacob find no difference in the blood of the veins and arteries, and show conclusively that after a short time there cannot be. Their experiments show that there is no such thing as hyperleucocytosis in the veins alone, which could only occur as the result of a direct new formation of white cells in the circulating blood. Both Löwit and Goldscheider and Jacob deny that this takes place. They both find immediately after the hypoleucocytosis almost exclusively mononuclear cells. Gradually, however, during the course of the hyperleucocytosis, a decided preponderance of polynuclear arises. Goldscheider and Jacob also lay much stress on the fact that most of these cells are eosinophile, and hence consider that

they are not entirely formed from the new mononuclear, but are partly old cells which were lying in the bone-marrow ready for extrusion and have been brought into the circulation in increased numbers as the result of the chemical irritation of the injected material.

Löwit considers hyperleucocytosis to be entirely independent of chemotaxis, and believes that it is due solely to a previous impoverishment of the blood in leucocytes. He also believes that a hyperleucocytosis occurs every time after a part of the leucocytes have perished, because an increased influx of young leucocytes out of the blood-forming organs is thereby brought on, that is, hyperleucocytosis follows leucolysis, which may result from the presence of certain substances, as bacteriological products, in the blood. He found that in all cases immediately after the injection there was a decided diminution in the leucocytes, but that then a gradual increase took place, which developed as much more intensely as the previous diminution was great. Thus, according to Löwit, hypoleucocytosis and hyperleucocytosis are two processes which are inseparably connected, the second occurring as the result of the first and being impossible without it. Goldscheider and Jacob were able, however, by the use of repeated small injections, to produce a primary hyperleucocytosis. They also showed, by autopsies on animals killed during the stage of hypoleucocytosis that the leucocytes were accumulated in the capillaries of the lungs and not destroyed. Löwit denies chemotaxis any part in the production of leucocytosis, but recognizes, however, that he cannot explain how leucolysis causes such a marked discharge of young cells from the blood-forming organs. It is difficult to see on what grounds he can deny the influence of chemotaxic action upon this process.

Schultz[10] believes that in all conditions which are considered as hypoleucocytosis and hyperleucocytosis the white cells are neither diminished nor increased, but are merely distributed differently in the vessels. According to his experiments with protein and other injections, there is neither a destruction of leucocytes — Löwit's leucolysis — nor an absolute increase of the leucocytes from the blood-forming organs afterward. That is, he believes that the hypoleu-

cocytosis is merely due to the accumulation of the white cells in the central vessels with a corresponding diminution of those in the peripheral, the hyperleucocytosis being due to the reversal of these conditions, the cells simply being differently distributed. Goldscheider and Jacob, however, found that the cells which accumulated in the capillaries of the lungs remained there, even increasing in number during the early stage of the hyperleucocytosis, thus showing that there is no shifting of the cells, but an absolute increase. This increase is sufficient to counterbalance the first loss of cells from the general circulation and still far exceed the number normally there.

Goldscheider and Jacob[5] have performed a very large number of very careful injection experiments on rabbits, using extracts of various organs and bacterial products. Many of their results have been already noted. They made counts immediately after injection and then at frequent intervals. After injections into veins they found in one case a marked hypoleucocytosis in twenty-five seconds. On the average the hypoleucocytosis reached its minimum in five or ten minutes; then the number began to increase, the maximum hyperleucocytosis usually being reached in five hours, but often only after twelve to eighteen hours; the normal was again reached in twenty-four to forty-eight hours. After subcutaneous injections the results were the same, but slower in making their appearance, the minimum appearing in three or four hours, the earliest maximum in fourteen to eighteen hours. Autopsies on animals killed during hypoleucocytosis showed the capillaries of the lungs to be full of leucocytes; there were as many, or more, in them during the hyperleucocytosis. They also succeeded, by giving repeated small injections, in producing a primary hyperleucocytosis. In other cases, by using very large doses, they caused death while the hypoleucocytosis still persisted.

Müller,[11] Everard, DeMoor, and Massart,[12] and Canthack[13] have obtained similar results as regards hypoleucocytosis and hyperleucocytosis. Everard sums up his work as follows: The injection of bacterial cultures, living or dead, results first in a diminution of the circulating leucocytes, especially of those with multiple nuclei and granular pro-

toplasm. When the animal survives the infection, the stage of hypoleucocytosis is followed by one during which the polynuclear granular cells are very abundant; then the blood resumes its normal characteristics. The hyperleucocytosis is not typical in cases which succumb to the injection, or may be entirely wanting in those which die quickly. Sometimes, when the infection is prolonged, it is replaced by a series of oscillations.

Canthack made hourly examination of the blood after the injection of filtered cultures of vibrio Metchnikovi. He found, at first, during the rapid rise of temperature, a marked diminution of the leucocytes which persisted until the pyrexia passed its climax. Four to six hours after, the number was enormously increased, reached its height at the ninth hour, but persisted, though to a less degree, for forty-eight or seventy-two hours, or even longer.

As the result of their experiments, Goldscheider and Jacob conclude that the phenomena of leucocytosis are due to chemotaxic action. The first action is a repellent one on the leucocytes, and they are arrested and held in the lung capillaries, where they remain. The second is irritative—attractive—on the blood-forming organs, and the white cells already formed there are set free and an increased number of new ones formed and also set free. Some of these also are arrested in the lungs as the result of continued repellent action. There is no instant leucolysis, and the process is something more than a mere changing of leucocytes, without increase, from the inner vessels to the outer, and *vice versa*. They explain the hyperleucocytosis without hypoleucocytosis which occurs after small doses by the fact that the small quantity, being distributed throughout the whole circulating fluid, is not sufficiently concentrated to exert an energetic enough repellent action on the leucocytes to cause much retention in the capillaries. If, however, such small doses are repeated at certain intervals, the positive, or attractive, influence gradually increases, finally becoming far superior to the negative influence which is exerted by these small doses. Thus the equilibrium between the two processes is finally destroyed in favor of the first, the result being a decided hyperleucocytosis. They

explain death during the hypoleucocytosis by the continuation of the repellent action, the irritative action being so strong as to paralyze the blood-forming organs.

Several difficulties confront us when we attempt to compare the leucocytosis in man with that observed in animals. We naturally cannot subject a sick man to so frequent blood-examinations as an animal; and as the disease often extends over days and weeks, important changes may easily escape observation. Moreover, we almost never get a blood-examination in the early stage of a disease. However, the vast number of blood-examinations which have been made clinically during the past few years have given us a general idea of the leucocytosis in most diseases. A hyperleucocytosis has been demonstrated in most acute infectious diseases, especially febrile.

A constant diminution has been found only in typhoid, malaria, and puerperal sepsis. There is said to be a decided hypoleucocytosis in the acute infectious diseases where the patients die within a few days. This is certainly the case in pneumonia. I have not observed it in diphtheria, the only case which had a normal number of white cells recovering.

It is possible that there may be a primary hypoleucocytosis in man, but, as already noted, the patient is almost never seen early enough to demonstrate this, if it exists.

It has been shown above, however, that it is possible experimentally to produce a primary hyperleucocytosis. Now the conditions in man are essentially the same as those in the experiments made regarding this point. For the ordinary method of infection is not by the sudden entrance of a large amount of toxic substances into the circulation, but by the continuous absorption of small quantities. Hence we expect a hyperleucocytosis without a previous hypoleucocytosis. If the infection be extremely sudden and severe and rapidly fatal, we have a condition analogous to that in animals killed by a very large dose during hypoleucocytosis. That is to say, the toxic material is sufficiently concentrated to paralyze the blood-forming organs. This would explain the fatal cases of pneumonia with no increase of white cells. Thus it is evident that the chemotaxic theory explains satisfactorily the leucocytosis found in man as well as in animals.

A series of experiments was made on animals to determine primarily whether the hyperleucocytosis observed clinically in diphtheria was due to the Klebs-Löffler bacillus and its toxines, or to associated bacteria. As far as I know, these are the first injection experiments ever made on animals with this bacillus or its products. At the same time a careful study of the leucocytosis was made, and the work of previous investigators in this line gone over. Autopsies were made in all cases and the organs examined.

Rabbits were used, as their ears naturally fit them for intravenous injections and the blood is easily obtained. The normal number of white corpuscles averages a little higher than in man. As there is a considerable variation in individual animals, a count was made in every case before beginning an experiment. Rabbits offer the further advantage that they present no digestion leucocytosis.[b] The blood was always taken from the ear and diluted (1 to 200) with a three-per-cent. salt solution colored with methylene blue. The Thoma-Zeiss instrument was used, and the corpuscles in at least fifteen cubic millimetres counted. The differential counts were of 500 white cells hardened with alcohol and ether and stained with Ehrlich's "triple-stain." The toxines used were from a filtered bouillon culture which had been twenty-three days in the thermostat, and was preserved by the addition of one-half per cent. of carbolic acid.

I wish here to acknowledge my indebtedness to Dr. J. H. Wright, of the Harvard Medical School, for the use of the toxine solution, and to thank both him and Drs. Councilman and Mallory for valuable advice and assistance in my work.

The object of the first experiment was to determine whether a local diphtheria would cause a hyperleucocytosis.

RABBIT NO. 1.

October 24. 9.30 A.M., white corpuscles, 5,800. 11 A.M., the palpebral conjunctiva of the left eye was excoriated over a small area, and a pure, fresh culture of the Klebs-Löffler bacillus on blood-serum rubbed in. This culture was the second generation from a fatal case.

October 25. 9.30 A.M., a spot of membrane, about one-quarter of an inch long and one-sixteenth of an inch wide, was present at the site of the excoriation. White corpuscles, 6,400.

October 26. 10.30 A.M., membrane all gone. White corpuscles, 7,100. As the amount of membrane had been so small, and the increase

of white corpuscles so slight as to be within the bounds of possible error, it seemed best to repeat the experiment. Accordingly, the conjunctiva was again excoriated at the same spot, but more deeply and over a larger area. 11.30 A.M., reinoculated with same culture.

October 27. 10.15 A.M., excoriated spot covered with membrane over an area about one-quarter of an inch square, the membrane also extending a little on to the ocular conjunctiva which had not been irritated. White corpuscles, 9,100.

October 28. 9.15 A.M., membrane a little less extensive. White corpuscles, 9,100. A blood-serum tube, inoculated from the eye at this time, twenty-four hours later showed a pure culture of the Klebs-Löffler bacillus.

October 29. 12 M., membrane only about half as extensive. White corpuscles, 8,400.

October 30. 9.30 A.M., membrane disappeared. White corpuscles, 8,200.

November 1. 9.30 A.M., white corpuscles, 6,000.

The result of this experiment proves that a local diphtheria, produced by the Klebs-Löffler bacillus alone, is capable of causing a hyperleucocytosis. The results also agree in a general way with the conclusions arrived at clinically: namely, that the hyperleucocytosis varies with the amount of membrane and ceases soon after its disappearance.

A series of experiments was next undertaken to determine if the toxine produced by the Klebs-Löffler bacillus would alone, as seemed probable, cause a hyperleucocytosis.

RABBIT NO. 2.

October 31. 9.30 P.M., white corpuscles, 12,400.

November 1. 10 A.M., one-half a cubic centimetre of toxine solution injected into ear-vein.

November 2. 9 A.M., found dead. A count of the heart's blood showed white corpuscles, 23,400. Autopsy showed inguinal, axillary, mesenteric, and retroperitoneal glands enlarged and reddened. Liver, macroscopically, showed no necrosis. Kidneys moderately congested. No fluid in thorax. Lungs somewhat congested and slightly edematous. Bone-marrow somewhat reddened.

RABBIT NO. 3.

November 2. 12 M., white corpuscles, 6,700. 12.45 P.M., one-tenth of a cubic centimetre of toxine solution injected into ear-vein.

November 3. 10.30 A.M., white corpuscles, 12,000.

November 4. 10 A.M., white corpuscles, 16,300. At this time the animal was decidedly sick, showing marked paresis of hind legs and some of front. 3.30 P.M., white corpuscles, 20,800. The rabbit was moribund at the time of this examination.

November 5. 12 M., autopsy, rabbit having died during the night, showed axillary glands enlarged and reddened. Little or no enlargement and no injection of inguinal, mesenteric, or retroperitoneal glands. Suprarenals much reddened. No macroscopic necrosis of liver. Kidneys much congested. No fluid in thorax. Lungs slightly congested, but not edematous. Bone-marrow very red.

In this case the leucocytosis had doubled in twenty-two hours and continued to increase steadily up to the time of death.

RABBIT NO. 4.

November 6. , 3.30 P.M., white corpuscles, 5,200. 4.30 P.M., one-fiftieth of a cubic centimetre of toxine solution injected into ear-vein.

November 7. 9.30 A.M., white corpuscles, 8,400. 3.30 P.M., white corpuscles, 11,400. The rabbit was now evidently sick.

November 8. 9.15 A.M., white corpuscles, 11,700. Rabbit much sicker. A differential count of the white cells made at this time, the height of the hyperleucocytosis, resulted as follows:

	Per cent.
Small Lymphocytes	30.8
Large Mononuclear and Transition Forms	5.8
Polynuclear Neutrophiles	7.8
Eosinophiles	55.6

The consideration of this, as well as of the following differential counts, will be taken up later.

November 9. 9.30 A.M., white corpuscles, 7,600. The rabbit was livelier than during the two previous days, and evidently on the road to recovery.

DIFFERENTIAL COUNT.

	Per cent.
Small Lymphocytes	41
Large Mononuclear and Transition Forms	10
Polynuclear Neutrophiles	11
Eosinophiles	38

November 10. 9.30 A.M., white corpuscles, 4,000. Rabbit still improving.

November 17. 9.30 A.M., white corpuscles, 5,800. The rabbit was now entirely well, but showed considerable induration about the seat of injection. It showed no signs of paralysis at any time.

In this case the increase in white corpuscles was well marked in seventeen hours, reached its maximum in forty-one hours, and had disappeared in eighty-nine hours. It is to be noted that the animal's condition varied with the

leucocytosis, he being sickest when it was at its height, and improved as it diminished. It is also worth noting that the hyperleucocytosis disappeared before any local reaction manifested itself, which is in direct contradiction to v. Limbeck's theory.

The three preceding experiments seem amply sufficient to prove that the toxines alone are competent to produce a hyperleucocytosis. They also justify the conclusion that the hyperleucocytosis in diphtheria is due to the absorption of the toxic products of the Klebs-Löffler bacillus and varies directly with the amount of absorption.

It is probable, however, that clinically the toxic products of associated bacteria play a part in its production. The subsequent experiments, although undertaken for other purposes, emphasize the same points.

The next experiment was undertaken to determine whether the toxines of the Klebs-Löffler bacillus cause an initial hypoleucocytosis, and if so, the time of its appearance and its duration.

RABBIT NO. 5.

November 10. 4 P.M., white corpuscles, 7,900.

DIFFERENTIAL COUNT, NORMAL BLOOD.

	Per cent.
Small Lymphocytes	45.2
Large Mononuclear and Transition Forms	6.6
Polynuclear Neutrophiles	11.2
Eosinophiles	37.0

November 11. 9.15 A.M., four-tenths of a cubic centimetre of toxine solution injected into ear-vein. 9.30 A.M., white corpuscles, 5,100.

DIFFERENTIAL COUNT.

	Per cent.
Small Lymphocytes	32.2
Large Mononuclear and Transition Forms	7.6
Polynuclear Neutrophiles	8.9
Eosinophiles	51.3

10.30 A.M., white corpuscles, 4,900.

DIFFERENTIAL COUNT.

	Per cent.
Small Lymphocytes	31.2
Large Mononuclear and Transition Forms	7.4
Polynuclear Neutrophiles	11.2
Eosinophiles	50.2

11.45 A.M., white corpuscles, 7,300. 3.30 P.M., white corpuscles, 13,100.

November 12. 9.15 A.M., white corpuscles, 11,500. The animal at this time, although not well, showed no very marked symptoms.

November 13. 9 A.M., found dead, but warm.

At the autopsy the axillary glands were slightly enlarged, but not much reddened. No enlargement or injection of inguinal, mesenteric, or retroperitoneal glands. Suprarenal capsules moderately reddened. No macroscopic or microscopic necrosis of liver. Kidneys much congested. No fluid in thorax. Lungs moderately congested, but not edematous. Bone-marrow very red.

As is evident above, the result of this experiment is positive, a definite hypoleucocytosis being present fifteen minutes after the injection. The lowest point was reached in an hour and a quarter. One and one-quarter hours later the number had nearly returned to normal, and three hours after this there was a marked hyperleucocytosis. The differential counts will be considered later. In "Rabbit 7," also, a marked hypoleucocytosis was present in half an hour.

The existence of hyperleucocytosis and hypoleucocytosis having been demonstrated, a series of investigations was begun to determine, if possible, the nature of the processes, and to determine, also, which, if any, of the theories detailed above was tenable. The best way of getting at this seemed to be to kill animals during the various stages, and then to make careful microscopic examinations of the organs, paying especial attention to the distribution of the white cells in them and to the bone-marrow.

The animals were killed by a sharp blow on the back of the neck, immediately autopsied, and the organs removed with as little handling as possible and placed in a saturated solution of corrosive sublimate in normal salt solution. After hardening in this for twenty four hours, they were washed in running water for twenty-four hours, then placed in absolute alcohol and mounted in celloidin in the usual manner. The sections were all cut of the same thickness in order to avoid error. Sections of the femur were made in the normal rabbit and in one killed during hyperleucocytosis, and kept in absolute alcohol for ten days. The outside bone was then removed, and the marrow placed in five-per-cent. nitric acid for twenty-four hours. It was then washed and

mounted in celloidin as were the other organs. Cover-slips were also made from the fresh bone-marrow and hardened in alcohol and ether.

RABBIT NO. 6.

A normal rabbit killed for purposes of comparison. It is noteworthy that the bone-marrow of the rabbit is extraordinarily rich in eosinophile cells, they forming the very great majority of the white cells present there.[14]

RABBIT NO. 7.

Killed during hypoleucocytosis.

November 15. 10.30 A.M., white corpuscles, 7,300. 11.15 A.M., seven-tenths of a cubic centimetre of toxine solution injected into ear-vein. 11.45 A.M., white corpuscles, 4,500. 12.15 P.M., killed.

At the autopsy no microscopic changes were recognizable. Bone-marrow, yellowish-red. Microscopical examination of specimens of the liver and kidney, stained with saffranin, showed no abnormal changes. There was no congestion and no increase in the number of white cells in their capillaries. Sections of the lungs were stained both with saffranin and with Ehrlich's "triple-stain," the latter giving a most beautiful differentiation of the white cells. These sections were carefully compared with those of the normal lung prepared in the same way. It must be admitted here that the conclusions arrived at are founded entirely on individual estimation and judgment, it being impossible to count the cells.

Drs. Councilman and Mallory, however, who were shown the specimens from the three cases, without being told which was which, arrived at the same conclusions regarding the number of white corpuscles as myself. While there was no noticeable increase in the number of red corpuscles in the lung capillaries, the white cells were very much more abundant than in the normal lung. In the large blood-vessels, however, the white corpuscles were much diminished. It was impossible to tell whether any one kind of white cells was more than proportionately abundant, the general impression being, however, that the proportions were about normal. Numerous hyaline thrombi were present in the lungs.

RABBIT NO. 8.

Killed during hyperleucocytosis.

November 18. 11 A.M., white corpuscles, 12,700. 11.45 A.M., two-tenths of a cubic centimetre of toxine solution injected into ear-vein.

November 19. 9.30 A.M., white corpuscles, 29,200.

Differential Count.

	Per cent.
Small Lymphocytes	15.4
Large Mononuclear and Transition Forms	1.6
Polynuclear Neutrophiles	4.4
Eosinophiles	78.6

11 A.M., killed

At the autopsy the axillary, inguinal, mesenteric, and retroperitoneal glands were unchanged. Suprarenals much reddened. No macroscopic necrosis of liver, which was somewhat congested. Kidneys much congested. Urine not hemorrhagic. No fluid in thorax. Lungs moderately congested, but not edematous. Bone-marrow yellowish red, but no redder than normal.

Microscopic examination of sections of the liver, stained with saffranin, showed marked congestion. The increase in white cells did not seem out of proportion to the increase in red, when the hyperleucocytosis was taken into consideration. Double-staining with eosin and methylene-blue failed to show any necrotic areas. Sections of the kidney, stained with saffranin, showed an enormous congestion. The increase in white cells, however, was not out of proportion to the hyperemia and hyperleucocytosis. Sections of the lungs were prepared as in the previous cases. There was a decided increase in the number of red cells in the capillaries. The white corpuscles seemed less abundant in the capillaries than during hypoleucocytosis. They were more numerous than in the normal lung, however, and seemed to be in larger numbers than the congestion and general hyperleucocytosis would explain. The impression obtained was that the eosinophile cells were more than proportionately abundant. The white cells in the large blood-vessels seemed about proportionate to the red corpuscles, when the general hyperleucocytosis was taken into consideration. Numerous hyaline thrombi were present in these lungs also.

Specimens of the bone-marrow stained with saffranin and compared with the normal showed a diminution in the number of polynuclear cells and giant cells: nuclear figures, however, were much more abundant. Those stained with "triple-stain" also showed a diminution in the number of giant cells. There was a great increase, however, in the number of small cells with small, round, deeply staining nuclei and a marked increase in the nuclear figures in these same cells. I am unable to give any explanation of the significance of these cells or of the change in them.

The object of the last experiment was to determine whether there was the same increase in the number of erythrocytes during experimental hyperleucocytosis as appeared clinically. The corpuscles in five cubic millimetres were counted at each examination.

RABBIT NO. 9.

December 1. 3.45 P.M., red corpuscles, 6,075,200; white corpuscles, 8,800; white to red, 1-690. 4.15 P.M., two-tenths of a cubic centimetre of toxine solution injected into ear-vein.

December 2. 9.30 A.M., red corpuscles, 7,436,400; white corpuscles, 12,400; white to red, 1-600.

That is, there was an increase in the number of red corpuscles of a little more than twenty per cent., thus confirming the clinical observations. As stated, when considering the clinical examinations, I am unable to find any satisfactory explanation for this increase.

December 3. 10 A.M., rabbit moribund. Circulation so feeble that no blood could be obtained from ear.

December 4. 9 A.M., died during the previous afternoon.

At the autopsy the axillary glands were moderately enlarged and reddened; inguinal glands less so; mesenteric and retroperitoneal not at all. Suprarenals moderately reddened. Liver moderately congested, but showed no macroscopic necroses. Kidneys much congested. No fluid in thorax. Lungs moderately congested and edematous. Bone-marrow no redder than normal.

The results of my experiments coincide pretty closely with those obtained by Goldscheider and Jacob. They show that hyperleucocytosis occurs without local inflammation or exudate, and that, experimentally at any rate, hyperleucocytosis is preceded by hypoleucocytosis. The presence of an increased number of white cells in the lung capillaries during hypoleucocytosis, and to a less extent during hyperleucocytosis, is in direct contradiction to Löwit's theory of leucolysis. The absence of an increase of white cells in the liver and kidney during hypoleucocytosis and the persistence of an increased number in the lung capillaries during hyperleucocytosis prove that Schultz's supposition that hypoleucocytosis and hyperleucocytosis are merely the expression of a shifting of leucocytes is wrong.

As already noted, my results were the same as those obtained by Goldscheider and Jacob, except that I found rather fewer leucocytes in the lung capillaries during hyperleucocytosis than during hypoleucocytosis. This is a comparatively unimportant difference, however, as there are so many more there than in the normal lung that it is evident that at least only a part of the cells can have returned to the general circulation, and hence that the white cells in the general circulation during hyperleucocytosis must be largely

new accessions from the blood-forming organs; that is to say, my results corroborate the chemotaxic theory and seem explicable by it alone.

Everard concludes that there is a special diminution in the polynuclear cells with granular protoplasm during hypoleucocytosis, and that these same cells are very abundant during hyperleucocytosis. Löwit and Goldscheider and Jacob found almost exclusively mononuclear cells immediately after hypoleucocytosis, and that a decided preponderance of polynuclear cells arises during the course of the hyperleucocytosis. The proportions of the white corpuscles in the rabbit differ decidedly from those in man. The average of the differential counts made in the normal rabbits is as follows:

	Per cent.
Small Lymphocytes	43
Large Mononuclear and Transition Forms	8
Polynuclear Neutrophiles	11
Eosinophiles	38

That is, there are twice as many small mononuclear cells as in human blood, while the polynuclear neutrophiles, which make up the bulk of the white cells in human blood, here form but a small factor. The eosinophile cells, however, are very much more numerous, making more than a third of the total. They probably correspond to the polynuclear neutrophiles in the human blood and represent the oldest forms. Attention has already been called to their extreme abundance in the normal marrow. Two counts made during hypoleucocytosis (Rabbit 5) resulted as follows:

	Per cent.	
Small Lymphocytes	32.2	31 2
Large Mononuclear and Transition Forms	7.6	7.4
Polynuclear Neutrophiles	8.9	11.2
Eosinophiles	51.3	50.2

That is, there was a diminution in the small mononuclear cells and a proportionate increase in the polynuclear eosinophiles, which is directly the opposite of Everard's observation. No count was made at the beginning of hyperleucocytosis. One made at its height (Rabbit 8) resulted as follows:

	Per cent.
Small Lymphocytes	15.4
Large Mononuclear and Transition Forms	1.6
Polynuclear Neutrophiles	4.4
Eosinophiles	78.6

Another made during the subsidence of hyperleucocytosis was as follows:

	Per cent.
Small Lymphocytes	30.3
Large Mononuclear and Transition Forms	5.8
Polynuclear Neutrophiles	7.8
Eosinophiles	55.6

This agrees entirely with the results of other observers, and is undoubtedly the analogue of the increase in polynuclear neutrophiles in man. Goldscheider and Jacob lay much stress on this large proportion of eosinophiles, and consider that they are not entirely formed from the new mononuclear cells, but are partly old cells which were lying in the bone-marrow ready for extrusion, and have been brought into the circulation in increased numbers by chemotaxic action. A careful study of slips made from the fresh bone-marrow was very unsatisfactory. The impression obtained, however, was that the eosinophile cells were less plentiful in the marrow of hyperleucocytosis than in the normal. As mentioned above, the cut section of the marrow of hyperleucocytosis showed a diminution in the number of polynuclear cells. These observations, as far as they go, agree with Goldscheider and Jacob's position, that the increase in the eosinophiles is partly the result of the extrusion of the cells already formed in the marrow, and confirm the chemotaxic theory.

The gross pathological lesions observed in the five animals autopsied during hyperleucocytosis are of some interest. In one case there was no involvement of the lymph-glands. In all the others the axillary glands were enlarged and more or less congested, while the inguinal were affected in two, and the mesenteric and retroperitoneal in only one. The suprarenal capsules were involved in every case, being moderately reddened in three and much in two. The kidneys were enormously congested in four cases, and moder-

ately so in one. The liver was slightly congested in two, not at all in the remainder. No macroscopic necroses were observed, and none were found in the two cases examined microscopically. The thorax never contained fluid. More or less congestion of the lungs was present in every instance, however, and in two there was slight edema. The bone-marrow was redder than normal in three cases.

To sum up, the constant and most striking lesions were congestion of the kidneys, suprarenals, and lungs. More or less acute lymphadenitis was usually present, and occasionally, edema of the lungs and reddening of the bone-marrow. Necrotic foci in the liver and fluid in the thoracic cavity, which were found so frequently by Wright [15] in guinea-pigs dead of experimental diphtheria, were not observed.

When my work was nearly completed, a paper was published by Gabritschewsky [16] entitled "Du Rôle des Leucocytes dans l'Infection Diphterique." He investigated the general leucocytosis of diphtheria in children and rabbits, and, in addition, studied the local leucocytosis and the action of the antitoxin serum on the progress of the leucocytosis. His conclusions regarding the general leucocytosis, based on his observations on fourteen children and on rabbits, are as follows: There is always a hyperleucocytosis in diphtheria, which is greatest in fatal cases, and which progressively diminishes during convalescence and after injections of antitoxin. A progressive hyperleucocytosis in diphtheria justifies a bad prognosis, and the analysis of the blood gives useful information regarding the value of treatment. He found that the number of white cells ordinarily varied between 11,450 and 25,000, and in fatal cases, between 29,500 and 51,000.

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